Isotachophoresis

T. Hirokawa, Hiroshima University, Higashi-hiroshima Japan

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Introduction

Isotachophoresis (ITP) is one of the electrophoretic techniques useful for the analysis and isolation of ionic substances. The first successful analysis using this method was reported for alkali earth metals and amino acids by Longsworth in 1953. The use of glass capillaries was first reported by Martin and Everaerts in 1967 after the successful separation of metal cations using a glass capillary by Konstantinov and Oshurkova in 1963 and of carboxylic acids using a paper strip by Schumacher and Studer in 1964. Although various names had been used for this method, the name ITP was proposed by Haglund in 1970 and has been widely accepted. It is interesting to note that the root of the present capillary electrophoretic methods is ITP.

An ITP separation is due to the different electrophoretic mobilities of the sample components in a similar manner to the other electrophoretic techniques. However, ITP has some characteristic features which distinguish it from the other electrophoretic techniques. This article, summarizes the theoretical background of ITP separations and then describes the analytical and preparative equipment used. Finally, separation strategies are given together with typical examples of ITP.

Electrophoretic Mobility and Velocity

Effective mobility μ of an ionic substance in a solution can be expressed as a function of many factors as follows:

$$\mu = f(m_0, K_a, \text{pH}, T, \eta, \varepsilon, I, K_s, C_{comp}) \qquad [1]$$

where m_0 is the absolute mobility of a solvated ion, K_a the acid dissociation constant, pH the pH of the solution, T the temperature, η the viscosity of the solvent used, ε the dielectric constant of the solvent, I the ionic strength, K_s the stability constants of the complexes or ion pairs formed, and C_{comp} the concentration of the complex-forming agent or the ion pair-forming agent.

Eqn [1] shows that the effective mobility is a complex function of the properties of the sample ion, the solvent used and the coexisting ions. A basic idea of the electrophoretic separation is to vary the mobilities of the ions being separated by varying some of the factors in the above function.

The migration velocity of the ion $(v)_i$ can be expressed as:

$$v_i = \mu E \qquad [2]$$

where E is the potential gradient of the electrical field. Consequently the difference of the electrophoretic velocities among separands is the driving force of the electrophoretic separation.

The name of 'isotachophoresis' comes from the Greek for equal (*iso*, *iso*) velocity (*tacho*, *tachoz*) sample dragging (*phoresis*, *foreesqai*). Although this name characterizes its principle as described later, if the sample velocities were the same throughout the migration process, there would be no separation.

Principle of ITP

Operational Electrolyte System and Separation Principle

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Two different electrolytes (a leading and a terminating electrolyte) are used in ITP and this is the important point which distinguishes it from other electrophoresis method. A sample solution is injected at the boundary of the two electrolyte solutions as shown in **Figure 1(A)**. The leading electrolyte is usually a pH-buffered electrolyte containing leading ions (L) with the same sign as the sample ions and appropriate counterions with pH-buffering ability. Usually, Cl^- and K^+ or NH_4^+ are used as the leading ions because of their large mobilities. The terminating electrolyte contains terminating ions (T) together with appropriate counterions.

For successful ITP separations, the effective mobilities of A and B in **Figure 1** should fulfil the following relationship:

$$\mu_L > \mu_A > \mu_B > \mu_T \qquad [3]$$

At the initial stage of migration, a homogeneous mixed zone (A + B) is formed as shown in Figure 1(B), where A and B migrate with different velocities under the same potential gradient. In this case, v_A is greater than v_B in the mixed zone. A forms a pure zone in the leading side of the mixed zone, and

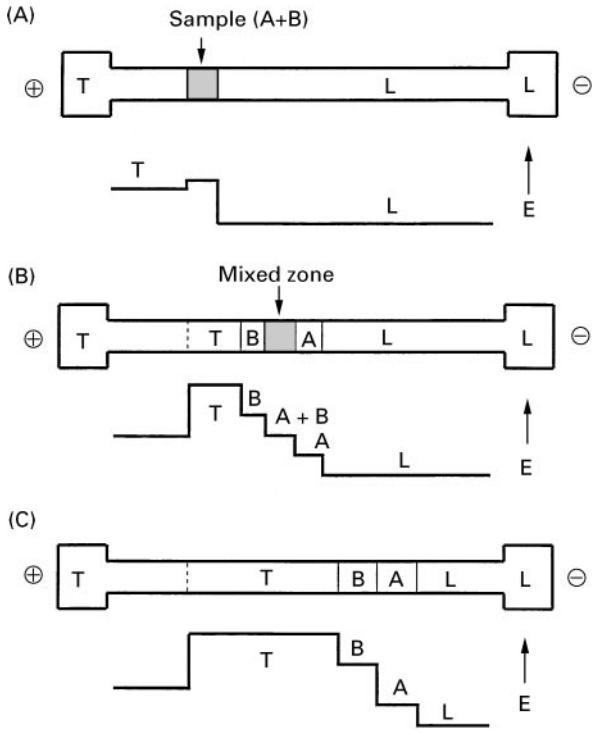

Figure 1 Separation process in isotachophoresis. (A) Before migration. (B) Separation process forming a mixed zone AB. (C) Complete separation (a steady state). L, leading zone; A, B, sample zone; T, terminating zone; E, potential gradient.

B forms a pure zone at the terminating side of the mixed zone. After a while, the mixed zone diminished and the sample components A and B are separated to form independent zones (**Figure 1**C). It should be noted that the migrating zones differ from those in zone electrophoretic migration. Once they are separated, they never mix again and the zone lengths are kept constant according to the sample amount as long as the migration current is applied. This is the isotachophoretic steady state.

When the migration order is as shown in **Figure 1**(C), the following relations are valid:

$$\mu_{A,A} > \mu_{A,B}$$
$$\mu_{B,A} > \mu_{B,B} \qquad [4]$$

where $\mu_{A,A}$ and $\mu_{B,B}$ denote the effective mobility of A and B ions in the steady state zone and $\mu_{A,B}$ and $\mu_{B,A}$ denote the effective mobility of A ions in the B zone and that of B ions in the A zone. This relation keeps the boundary between A and B zones very sharp (self-sharpening effect).

At the isotachophoretic steady state where the zone lengths of all samples are constant and no mixed zone remains, the following relations are valid for a constant current:

$$v_L = v_A = v_B = v_T \qquad [5]$$
$$E_L\mu_L = E_A\mu_A = E_B\mu_B = E_T\mu_T \qquad [6]$$

From eqns [3] and [6] the following relationships between the potential gradient of zones (Figure 1) apply:

$$E_L < E_A < E_B < E_T \qquad [7]$$

Therefore the separated zones can be detected by the use of a potential gradient detector and a conductivity detector besides spectroscopic detectors.

It should be noted that the pH of the ITP system is different in the different zones. The pH buffering counterions are continuously supplied from the leading electrolyte, and they regulate the pH of the sample zones. Usually the following relationship is valid for an anion analysis:

$$pH_L < pH_A < pH_B < pH_T \qquad [8]$$

The reversed relationship holds in a cationic analysis. The difference $|pH_L - pH_T|$ is usually less than 1 when the pH buffering counterion is selected properly. Since the pH of the zones are thus not constant in contrast to zone electrophoresis, the effective mobility dependence of the samples on the pH of the leading electrolyte cannot be estimated straightforwardly. And this makes the separation optimization of isotachophoresis difficult in comparison with zone electrophoresis.

The pH buffers conventionally used are summarized in **Table 1** for anion analysis. To keep good buffering capacity of the leading electrolyte, the pH_L

Table 1 pH buffers used for anionic analyses

Buffer	pK_a	*pH_L range*
Glycylglycine	3.140	2.6–3.6
β-Alanine	3.552	3.0–4.0
ε-Aminocaproic acid	4.373	3.8–4.8
Creatinine	4.828	4.2–5.4
Histidine	6.040	5.4–6.4
Imidazole	7.150	6.4–7.4
TRIS[a]	8.076	7.4–8.4
Ammediol[b]	8.780	8.2–9.2
Ethanolamine	9.498	9.0–10.0

[a]TRIS, tris(hydroxymethyl)aminomethane.
[b]Ammediol, 2-amino-2-methyl-1,3-propanediol. These buffers are used to adjust the pH of an electrolyte containing a leading ion such as hydrochloric acid.

should satisfy the following relationship:

$$pK_Q - 0.5 < pH_L < pK_Q + 0.5 \qquad [9]$$

where pK_Q is the pK_a of the buffers used. The maximum buffering capacity is obtained at $pH_L = pK_Q$.

The isotachophoretic steady state is achieved when the following four conditions are fulfilled:

- The leading and the terminating electrolyte are chosen properly to satisfy eqn [3].
- A constant migration current is applied.
- Mass balance of the counterion is kept: the molar amount of the pH buffer flowing into the sample zone in a unit time should be equal to the amount flowing out the sample zone.
- Electroneutrality is kept in each zone as in normal electrolyte solutions.

ITP is bidirectional in principle, since electrophoretic phenomena are bidirectional. In fact, isotachophoretic stacking zones can be formed simultaneously for anionic and cationic components in a sample when a suitable electrolyte system is chosen. In bidirectional ITP, the anolyte and the catholyte are the leading electrolyte and the terminating electrolyte for anions, and vice versa for cations.

Although the selection of the operational electrolyte system for bidirectional isotachophoresis is not too difficult, the difference between the pH of an anolyte and that of a catholyte is restricted.

Qualitative Analysis

The effective mobility of a sample component is uniquely determined under a given set of experimental conditions and this allows qualitative analysis. In practice, some qualitative indices have been proposed using some different definitions on the basis of the ratio of the potential gradients or the conductivities of the separated zones. We have proposed R_E, which is defined for the component A as follows:

$$R_{E,A} = E_A/E_L = \mu_L/\mu_A \qquad [10]$$

Figure 2 shows the experimental definition of R_E values using step heights, where the asymmetric potential of the potential gradient detector (δh) is corrected by the use of an internal standard. By comparing such qualitative values of samples with those of reference standards, tentative qualitative analysis can be done. Additional information by a UV/VIS online detector may be useful. For exact identification, ITP zones are fractionated and the fractions analysed by independent analytical methods.

Quantitative Analysis

In ITP, the concentration of ions in the steady-state zone is determined by their effective mobilities and by the concentration of the leading ion. Therefore, dilute components in a sample are concentrated according

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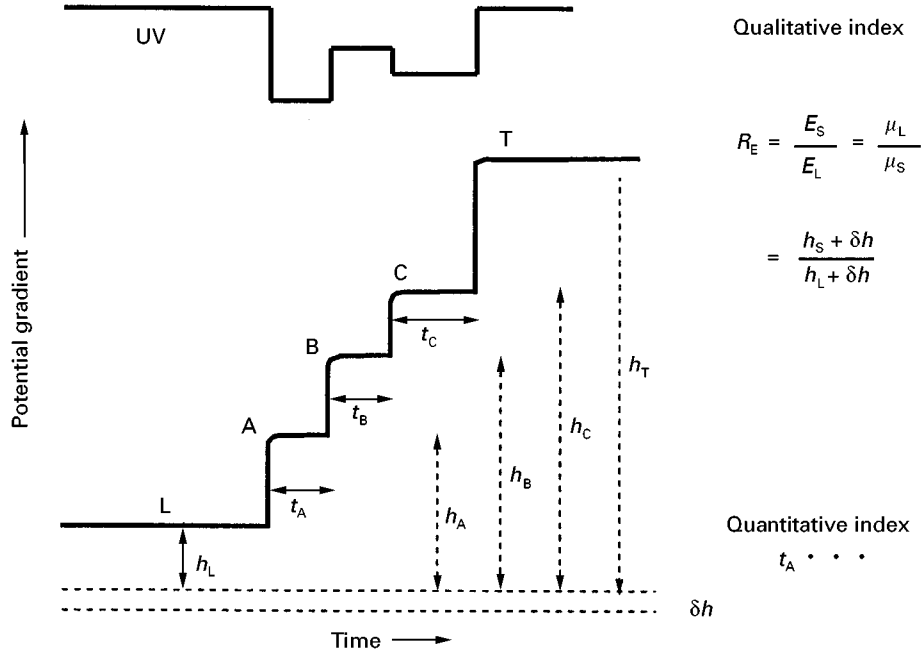

Figure 2 Isotachopherograms and experimental definition of R_E values. L, leading zone; A, B, C, sample zone(s); T, terminating zone; h, step height; δh, step height corresponding to an asymmetric potential of a potential gradient detector, which can be determined using theoretical R_E value of internal standard; R_E, corrected R_E value of sample. The subscript S denotes component A, B or C.

to Kohlrausch's regulating function and conversely the concentrated components are diluted during migration. The following relation is valid among the total equivalent concentrations of the samples (C^t):

$$C^t_L > C^t_A > C^t_B > C^t_T \qquad [11]$$

The quantitative index is the time-based zone length as shown in Figure 2. The absolute zone length of the sample A (l_A in cm) can be defined as:

$$l_A = 1000n/(C^t_A \pi r^2) \qquad [12]$$

where n is the amount of applied sample (moles) and r the radius (cm) of the separation tube at a detector.

The zone passing time, t (s) is equal to the actual zone length divided by the ITP velocity, v (cm s^{-1}). When both the sample ion and the buffer ion are monovalent, t_A can be expressed as follows:

$$t_A = Fn(1 + \mu_Q/\mu_A)/i \qquad [13]$$

where F is the Faraday constant, μ_Q the mobility of the buffer ion, μ_A that of sample A, and i the migration current.

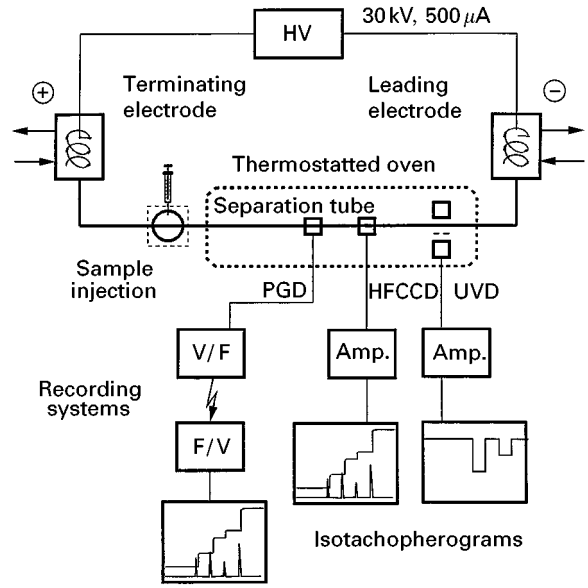

Figure 3 ITP apparatus with potential gradient detection (PGD), high-frequency contactless conductivity detection (HFCCD), and ultraviolet detection (UVD). HV, high-voltage power supply (constant current); V/F, voltage-frequency converter; F/V, frequency-voltage converter.

Instrumentation

Separation System for ITP Analyser

A typical diagram of ITP analyser is shown in **Figure 3** for unidirectional ITP. A separation tube connecting two electrodes is made of polytetrafluoroethylene (PTFE) or fused silica, whose inner diameter is 0.2–0.5 mm. A PTFE tube as large as 1 mm inner diameter is frequently used before detection as a pre-column to increase column hold up or electric charge for better resolution. An additional leading electrode chamber can be used to apply high current during pre-separation to reduce analysis time. The chamber is connected appropriately to the separation tube before the detector. The leading and the terminating electrode chamber are typically 20 mL in volume.

The separation tube is also filled with a leading electrolyte and a terminating electrolyte. The boundary is formed at a sample injection port and a sample solution is introduced there typically by using a microsyringe. Sample components are separated during migration and their zones are detected using appropriate detectors.

Detectors

The quality of the detector employed determines and limits the qualitative and quantitative analysis of ITP. There are two demands on the detector; firstly to accurately reflect the separation occurring in ITP and secondly to obtain the isotachopherogram from the analysis with high reproducibility.

Detectors for ITP can be divided into universal and specific types. The signal from universal detectors is directly proportional to the effective mobilities of the ionic species, and these detectors detect zones of all components separated in the narrow-bore tube. Thermometric, potential gradient and conductivity detectors belong to this class. The detection limit of potential gradient and conductivity detectors is subnanomole but that of thermometric detector is rather high. On the other hand, specific detectors such as UV spectrophotometers allow the identification of some components directly, or at least can give additional information about zones.

In **Figure 3**, three detection systems using a high-frequency contactless conductivity detector (HFCCD), a potential gradient detector (PGD), and an ultraviolet detector (UVD) are shown. Since the sensing electrodes of PGD directly contact the solution in the capillary, this system needs a device to isolate high voltage. In **Figure 3**, a photocoupler is used for this purpose (IP-1B, IP-2A, IP-3A, Shimadzu, Kyoto, Japan, production discontinued). A transformer is used in the usual (contact type) conductivity detection (a.c. method). Although the sensitivity of a contactless detector is lower than the direct contact

detector, the merit of HFCCD is obviously that the detection system needs no such isolation device.

Preparative ITP Apparatus

Capillary type ITP is useful not only for analytical purposes but also for preparative purposes. Capillary type (CITP) is useful for batch processing of a small amount of sample.

In addition to direct cutting of the capillary section containing the target of interest, preparative methods in CITP can be classified into three types, as shown in **Figure 4**. Figure 4(A) shows a preparative ITP system reported by Arlinger for the fractionation of the entire sample zones. This system was used in the LKB Tachofrac (Bromma, Sweden, 1983, production discontinued). The zones were swept gradually by a counterflow of a leading electrolyte (ex. 3 μL min^{-1}) on applying migration current, and the fractions were fixed on a cellulose acetate strip. The separated zones were successively pushed out through a T-branch by applying a counterflow and the zones were continuously fixed on the strip by an electric spray. The linear velocity of the counterflow was set only a few percent higher than the isotachophoretic migration velocity so as not to dilute the sample by the leading electrolyte. The fractions on the strip can be analysed by immunological and radioactive methods. The zymogram technique can be used directly on the strip. The fractions have to be eluted, for analysis by other methods.

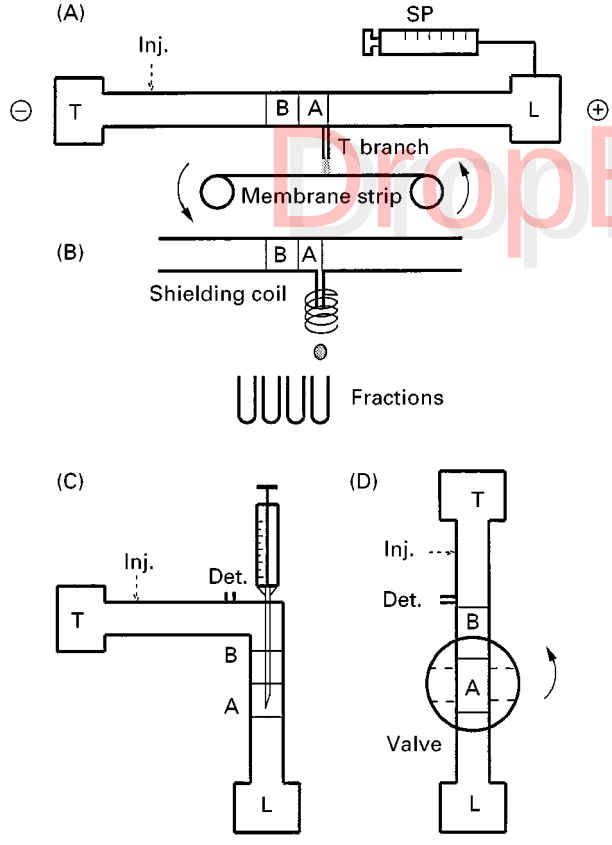

Figure 4 Preparative methods in capillary-type isotachophoresis. (A) Arlinger's counterflow method. (B) Modified Arlinger's method. (C) Microsyringe method. (D) Fractionation valve method. A and B, samples; L, leading electrolyte; T, terminating electrolyte; Inj., sample injection port; SP, a counterflow pump; Det., a detector.

A dropwise fractionating method was developed utilizing a counterflow technique. The schematic diagram of the apparatus is shown in **Figure 4**(B). When the sample zone is pushed out from a T-branch, a spray effect is usually observed due to electrostatic forces. This can be a convenient interfacing technique but it disturbs dropwise fractionation. The electric spray and fluctuation of the drop rate due to electrostatic forces are suppressed by a very simple electrostatic device: As shown in **Figure 4**(B), the exiting fraction is surrounded by a copper coil, which is connected to a nozzle. The fractions are collected directly into small test tubes on the fraction collector through the coil. By using this technique, complete recovery of the mobile components in the injected samples is possible with minimum risk of loss and contamination. It should be noted, however, that mixing of adjacent sample zones cannot be avoided. The average volume of one drop was ca. 5 μL and the deviation was estimated as ±10%. A few nanomoles of the sample components are contained in a drop. The concentration of samples in the fractionated drops or the amount of the target in a fraction was adjustable by changing the flow rate of the leading solution. A typical counterflow of a leading electrolyte was ca. 12 μL min^{-1}, which is much higher than the Arlinger-type apparatus.

Figure 4(C) shows another method reported by Kobayashi *et al.*, where the separated sample zones are discontinuously isolated by using a microsyringe. Kobayashi *et al.* used a potential gradient detector (PGD) with a sample-removal port to fractionate the target zone immediately after the tail of the zone was detected by the PGD. Although the method was not intended for the successive fractionation of the entire sample zones, the ease of operation is notable. This technique was employed for IP-1B and IP-2A instruments (Shimadzu, Kyoto, Japan, production discontinued).

Figure 4(D) shows other discontinuous fractionation technique using a specially designed fractionating valve placed at the end of the separation capillary. After trapping the target zone in the valve, the zone is flushed out.

In addition, the separation tube used was a series of four separation tubes (inner diameter of the tubes, 5–0.5 mm) in order to increase the amount of

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sample separated. The tube of 5 mm inner diameter was made of acrylic resin and the maximum injectable sample volume was 2.5 mL.

Free-flow apparatus Since no solid media are used in free-flow electrophoresis (FFE), the most important point in instrumentation is the stabilization of the separated zones for any electrophoresis mode. Unstable zones may be caused by unstable operational electrolyte, sample flow, heat convection, density-driven flow, electroosmosis, etc. Bier *et al.* and Thormann *et al.* summarized several different methods for stabilizing the zones. A flat-type FFE is treated here, although there are several different approaches using different geometries, such as a thin film between parallel plates, a cylindrical laminar flow between two coaxial cylinders, etc.

Continuous FFE apparatus utilizing a thin flowing fluid was originally designed by Hanig for zone electrophoresis. Prusik and Wagner *et al.* designed and constructed similar apparatus, suggesting that several modes of electrophoresis can be used. At present, the only FFE apparatus available is the Octopus continuous electrophoresis apparatus from Dr. Weber GmbH (Kirchheim-Heimstetten, Germany). By using this apparatus, up to several grams of pure substances can be prepared daily, although the amount depends on the properties of the sample. **Figure 5** illustrates the electrolyte circuits of an FFE system (Octopus)

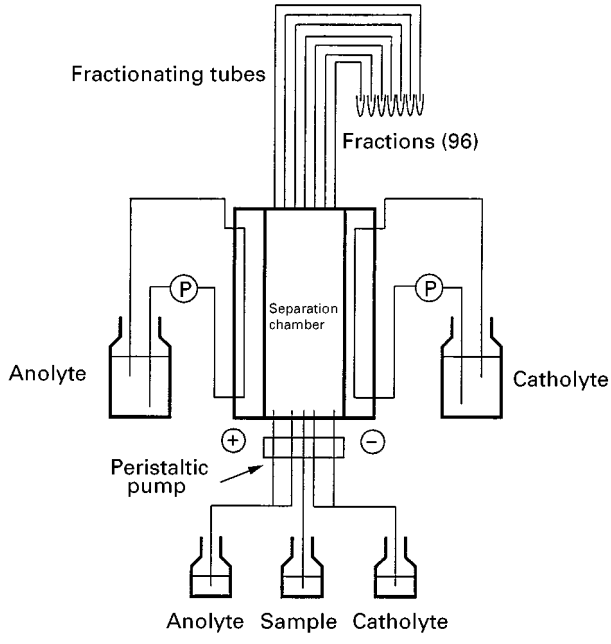

Figure 5 Electrolyte circuits of a free-flow electrophoresis apparatus (Octopus, Dr. Weber GmbH). Outer dimensions of separation chamber are $640 \times 180 \times 80$ mm, Separation area is 500×100 mm (variable thickness 0.4–2.0 mm). P, pumps.

when operated in continuous free-flow isotachophoresis (CFFITP) mode. The effective size of a typical separation chamber is 10-cm wide, 50-cm high and 0.4-mm thick. The sample solution is supplied with a multifold peristaltic pump together with an anolyte and a catholyte. Overflow of the separation chamber is collected as 96 fractions. The flow rate is variable in the range 0.3–100 mL h^{-1}. The sample residence time is variable in the range 1–40 min. High flow rate and small residence time allow stable flow and consequently stable position of zones.

The anolyte and catholyte are circulated by pumps during migration. A dialysis membrane isolates the separation chamber from the electrode compartments. The electrolyte solutions may be denatured (the pH will change) after a few hours operation. The separation chamber can be thermostatted and separation can be monitored with a VIS CCD detection system which can be positioned near the end of the chamber. To obtain pure fractions, the positions of the sample zones at the end of the sample chamber should be stable. The positions are dependent on several factors such as the electric field strength, temperature of the electrolytes, flow rate, and sample and buffer composition. Since these factors are closely correlated with each other, careful control is needed. Sufficient residence time and separation distance are necessary especially when mobility differences are small. For this purpose, a larger separation chamber or a counterflow technique should be used as reported by Prusik (a 50×50 cm square chamber with a thickness of 0.5 mm).

Separation Strategy and Typical Applications

Operational electrolyte conditions should be optimized to obtain the best quality of separation by changing electrolyte parameters so that the difference of the effective mobilities of the target components should be as large as possible. Strictly speaking, the separability depends on the mobility difference in the mixed zone (see **Figure 1**). However, to a first approximation, the mobility differences at the steady state or the difference of the R_E values may be used for the criterion for optimization of separations.

For separation optimization in electrophoresis, a theoretical approach is sometimes very useful. In isotachophoresis especially, the pH and the ionic strength of the separated zones are different to one other. The optimum electrolyte system can be determined by iterative computer calculations where the effective mobilities of the species being separated at the steady state are calculated using their

physicochemical constants, and the differences of the effective mobilities compared. This method may be called computer-aided separation optimization, which enables the determination of the electrolyte conditions such as the pH without time-consuming 'trial-and-error' experiments. Some examples of simulation are also included in this section.

pH Effect

When the sample contains weak electrolytes, the pH of the leading electrolyte (pH_L) should be carefully chosen to obtain high separability among the components, since the effective mobilities of weak electrolytes change drastically according to the pH of the solution. This pH effect on the effective mobility is the most important and should be examined first.

Figure 6 shows the simulated and the observed isotachopherograms for a test mixture of pyrophosphoric (P_2O_7), triphosphoric (P_3O_{10}), tetraphosphoric (P_4O_{12}) acids, and 15 nucleotides, AMP, ADP, ATP, CMP, CDP, CTP, GMP, GDP, GTP, IMP, IDP, ITP, UMP, UDP and UTP. The pH_L was adjusted to 4.7 with creatinine. The optimum pH_L range was rather limited, because CTP may form a mixed zone with UDP at low pH_L and with ATP at the high pH_L. Obviously from this example, the pH of the leading electrolyte should be carefully chosen in order to obtain high separability for weak electrolytes. For this purpose, computer simulation of the isotachophoretic steady state is useful, when the mobility and dissociation constants are available. In fact, the optimum pH_L of the above separation has been determined by simulation.

Solvent Effect

Since the mobility is strongly affected by the viscosity of the solvent and the dissociation constants depend on the dielectric constant of the solvent, the use of a nonaqueous solvent or a mixed solvent may improve electrophoretic separations (the solvent effect). The other advantage of the use of a nonaqueous systems is that it enables the analysis of substances with low water solubility. Many solvents have been successfully applied for isotachophoresis, such as methanol, ethanol, dioxane, acetone, propanols, dimethylformamide, etc. The migration behaviour in a nonaqueous solvent is very different from that in an aqueous solvent. The use of a mixed solvent

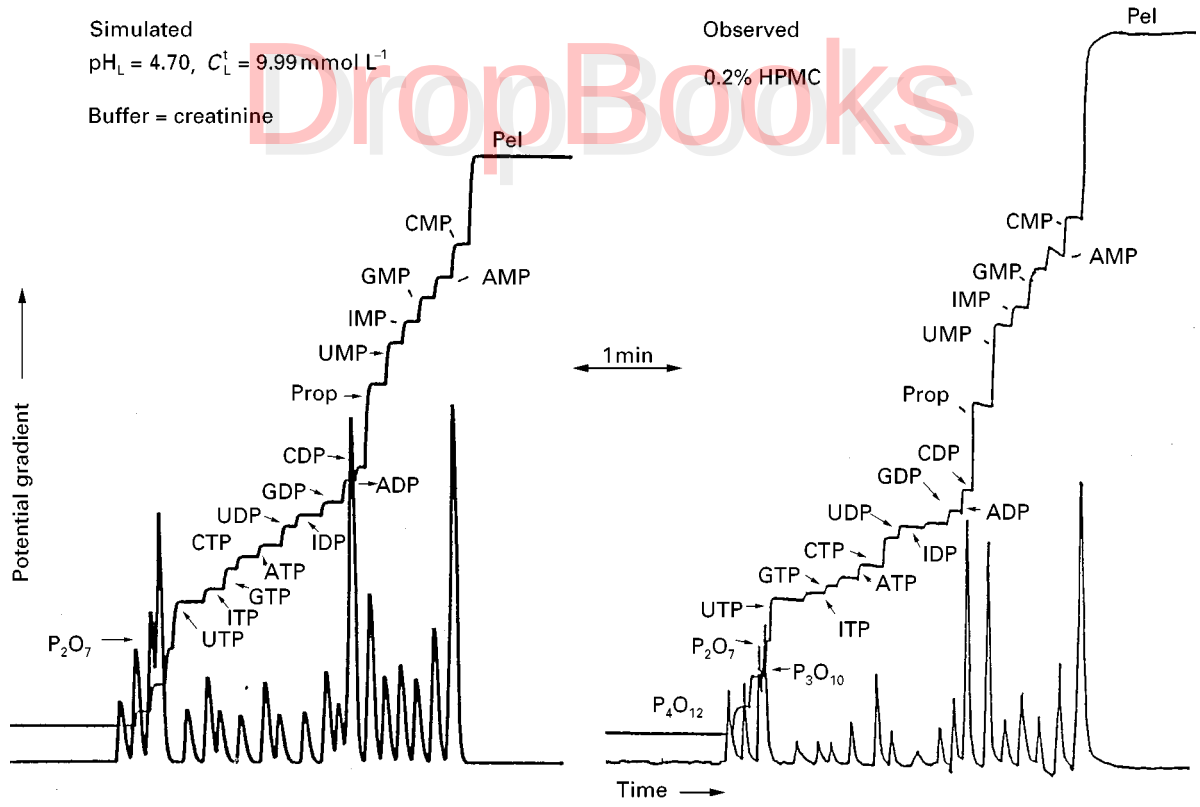

Figure 6 Simulated and observed isotachopherograms of 19 anions, AMP, ADP, ATP, CMP, CDP, CTP, GMP, GDP, GTP, IMP, IDP, ITP, UMP, UDP, UTP, pyrophosphoric acid (P_2O_7), triphosphoric acid (P_3O_{10}), tetraphosphoric acid (P_4O_{12}) and propionic acid (Prop) at $pH_L = 4.7$ (creatinine buffer). The terminator was pelargonic acid (Pel). Current, 75 μA. Sample amounts, 1.2–3 nmol. The leading electrolyte contained 0.2% hydroxypropylmethylcellulose (HPMC). Buffer, creatinine.C_L^t, concentration of leading ion.

(aqueous–nonaqueous) provides further possibilities for mobility control to improve separations.

Figure 7(A) and (B) show the simulated and observed isotachopherograms at pH 8 using triethanolamine buffer when methanol is used as solvent. The leading ion is perchlorate. Obviously, the separation is complete. On the other hand, insufficient separation was predicted for the aqueous system where the leading ion was chloride when $pH_L = 3.6$ (β-alanine buffer), as shown in Figure 7(C). Apparently the step heights of 2-naphthalenesulfonate, picrate and *o*-chlorobenzoate were similar and they may form a mixed zone in the actual analysis. No satisfactory separation was estimated when pH effects in the aqueous system were used.

Thus it is evident that the separation behaviour in isotachophoresis is strongly affected by the solvent used. As demonstrated above, high separability can be expected by the use of methanol solvent for particular samples but it should be noted that carboxylic acids are esterified gradually on standing in methanol solution, although the production of esterified compounds during analysis is negligible.

Complex-forming Effect

The use of a complex-forming agent is a traditional technique to improve separability especially in the case of metal ions. To achieve complex-forming equilibria, a constant amount of the complex-forming agent should be supplied continuously to the sample zone. The complexing agent can be supplied as the counterion in the leading electrolyte, as neutral ligand (crown ether and cyclodextrin), or as the terminating ions.

Complex formation is very useful for the separation of metal ions. A typical example of the isotachophoretic separation of metal ions utilizing the complex-forming effect was reported for lanthanides, where α-hydroxyisobutyric acid (HIBA) was used as the complexing agent. The concentration of the complexing agent and the pH of the leading electrolyte should be optimized to obtain a good separation. By adding malonic acid to the main agent HIBA, 15 rare-earth ions (lanthanide and yttrium) were successfully separated as shown in **Figure 8**.

Conclusion

Isotachophoresis is a useful analytical technique with high reproducibility. However, ITP is not a very familiar technique to many chromatographers. Possible reasons are absolute sensitivity of ITP is in the subnanomole range, which is low in comparison with recent capillary electrophoresis (CE) techniques; auto-samples are not available; and stepwise recording is not readily accepted by chromatographers. However, it should be noted that the relative sensitivity of ITP is comparable with CE especially when the detection method is the same (e.g. UV detection), since a relatively large volume of a sample (e.g.

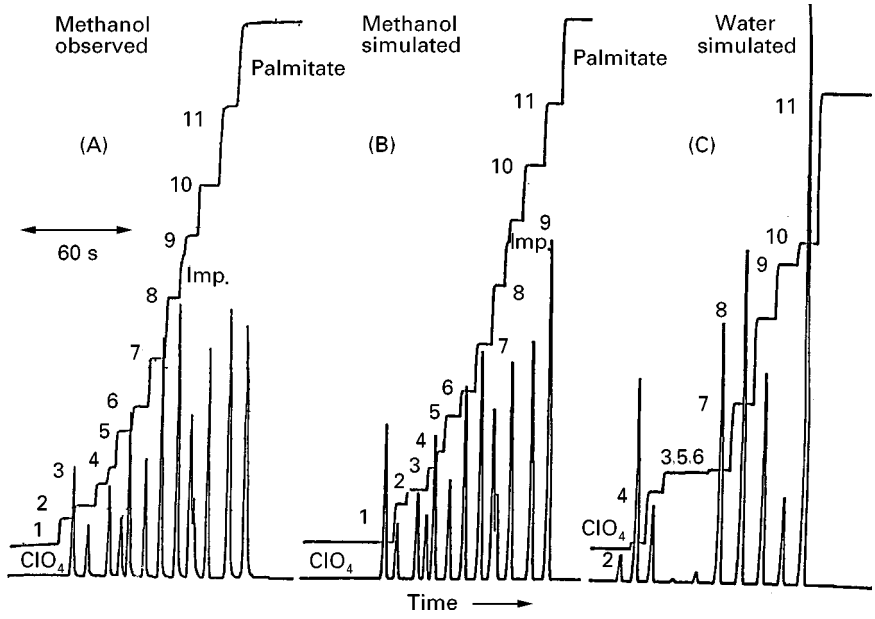

Figure 7 The observed (A) and the simulated isotachopherogram (B) of (1) bromide, (2) chloride, (3) picrate, (4) formate, (5) 2-naphthalenesulfonate, (6) *o*-chlorobenzoate, (7) *p*-chlorobenzoate, (8) 3-hydroxybutyrate, (9) crotonate, (10) propionate and (11) pelargonate ions in methanol as solvent. The leading ion was 10 mmol L^{-1} ClO_4^- and the buffer used was triethanolamine ($pH_L = 8.06$). (C) The simulated isotachopherogram of the above samples except for bromide. The leading ion was 10 mmol L^{-1} chloride and the buffer used was β-alanine ($pH_L = 3.6$). The terminator was pelargonate. Imp., impurity of the used electrolyte system.

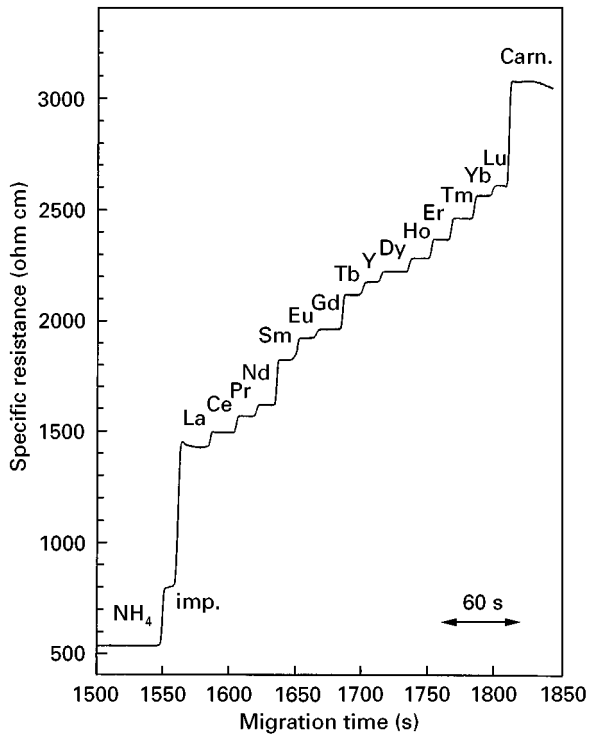

Figure 8 The observed isotachopherogram of 15 rare-earth ions (lanthanide ions and yttrium ion). HIBA, the complex-forming agent α-hydroxybutyric acid. The leading ion, 20 mmol L^{-1} NH_4^+; pH buffer = 2-ethyl-*n*-butyric acid ($pH_L = 4.8$). The sample amount was 0.33 mmol $L^{-1} \times 5$ μL. Migration current = 40 μA. The terminator is carnitine hydrochloride. (Carn.) imp., impurity of the used electrolyte system.

100 μL) can be injected. For preparative purpose, ITP is sometimes better than CE especially when the sample size is relatively large. In order to utilize the favourable features of ITP, an automated apparatus is needed or a method should be found to use commercial CE apparatus for ITP.

Further Reading

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Micellar Electrokinetic Chromatography

M.-L. Riekkola, Laboratory of Analytical Chemistry, University of Helsinki, Finland

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Introduction

Micellar electrokinetic capillary chromatography (MEKC), first introduced by Shigeru Terabe and coworkers in 1984, has extended the potential of